AMENDMENTS

In the specification

Page 1, lines 13-14, please replace the two blanks ("_____") with the U.S. Provisional Application numbers --60/031,306-- and --60/035,345--, respectively.

In the claims

C1

43. (Amended) The [vaccine] immunogenic composition of claim 40, further comprising an adjuvant.

59. (New) A composition comprising an effective amount of anti-idiotype antibody of claim 1, wherein an effective amount is an amount sufficient to elicit an anti-human milk fat globule immune response.

C2

60. (New) A composition comprising an effective amount of the antibody of claim 4, wherein an effective amount is an amount sufficient to elicit an anti-human milk fat globule immune response.

61. (New) A composition comprising an effective amount of the polypeptide of claim 20, wherein an effective amount is an amount sufficient to elicit an anti-human milk fat globule immune response.

REMARKS

Telephone interview

Applicants' representative wishes to thank Examiner Reeves for extending the courtesy of a telephone interview on September 25, 1998, and the helpful discussion that ensued. Applicants believe that the amendments and remarks are fully responsive to the issues raised by the Examiner.

At the interview, the following points were discussed:

- The Examiner suggested submitting declaratory evidence that neither the 11D10 antibody nor the hybridoma producing 11D10 were made available to the public.
- The Examiner suggested submitting declaratory evidence addressing the contributions of non-inventor co-authors in order to establish that the co-authors were not inventors.
- The Examiner suggested that Applicants' representative submit a declaration regarding the hybridoma deposit. The declaration, as well as a copy of the ATCC receipt, accompanies this preliminary amendment.

Status of the application and the claims

Claims 1-5, 20-24, 26, 35-37, 39, 40, 42, 43, 54-56 (Group I) are currently pending. A previous preliminary amendment was filed by facsimile on July 30, 1998. By the present amendment, claim 43 has been amended. New claims 59-61 have been added. Support for these claims is found in the specification on, inter alia, page 28, lines 19-26; page 40, lines 3-13; page 42, line 22 to page 43, line 25; page 86, lines 10-15; page 89, line 19 to page 90, line 26.

During the telephone interview of July 28, 1998, the Examiner indicated that amending claims 40, 42 and 43 to recite "immunogenic composition" rather than "vaccine". The specification defines "vaccine" as "a pharmaceutical composition for human or animal use, which is administered with the intention of conferring the recipient with a degree of specific immunological reactivity against a particular target, or group of targets. The immunological reactivity may be antibodies or cells (particularly B cells, plasma cells, T helper cells, or cytotoxic T lymphocytes and their precursors or any combination thereof) that are immunologically reactive against the target." Page 27, lines 11-19. In order to expedite

prosecution, these claims have been amended to recite "an immunogenic composition". Support for this amendment is found, inter alia, in the definition of "vaccine".

Request for rejoinder

Applicants respectfully request that, in accordance with well-established Office policy and *In re Ochiai*, that method claims incorporating all limitations of the product be rejoined upon allowance of the product claims.

Declaration regarding ATCC deposit

The Examiner suggested that Applicants' representative submit a declaration regarding the hybridoma deposit. The declaration, as well as a copy of the ATCC receipt, accompanies this preliminary amendment. Further, Dr. Malaya Chatterjee states in her declaration that the deposited cell line is that same as that described and claimed in the application (as well as previously filed provisional application 60/031,306, filed December 20, 1995).

Applicants respectfully request that the Office acknowledge on the record that the deposit information is complete.

Lack of anticipation by references co-authored by inventors

The Examiner has not articulated an anticipation issue with respect to certain scientific publications co-authored by the inventors. These references are: (a) Bhattacharya-Chatterjee et al., "Anti-idiotype antibodies as potential therapeutic agents for human breast cancer", in *Antigen and Antibody Molecular Engineering* (Ceriani, ed.) (1994), pages 139-148; (b) Bhattacharya Chatterjee et al., *Cancer Immunol. Immunother.* (1994) 38:75-82; (c) Chakraborty et al., *Proc. Am. Assoc. Cancer Res.* (1994), Abstract 2963; (d) Mukerjee et al. (1992) *Fed. Amer. Soc. Exp. Bio.*, Abstract 6505; (e) Mukerjee et al. (1991) *Fed. Amer. Soc. Exp. Biol.* Abstract 7792; (f)

Chakraborty et al. (1995) *Cancer Res.* 55:1525-1530. This is presumably due to the fact that the Examiner understands that none of these references are enabling for the claimed invention and that the probability of reproducing the 11D10 antibody by following only what is provided in any of these references is vanishingly low. However, for the record, Applicants present the following reasons why none of these publications anticipate the 11D10 antibody and do not affect patentability of (i.e., are not prior art with respect to) any of the pending claims.

Each of the following reasons why none of these publications anticipate the pending claims shall be discussed in turn: (a) the cited references are non-enabling because they do not teach and/or enable obtaining 11D10 antibody, and do not disclose the amino acid sequence or DNA coding sequence for the variable regions of 11D10, and thus cannot be used as a prior art reference; and (b) neither 11D10 nor the hybridoma producing 11D10 have been made available to the public.

(a) The references of record are non-enabling

It is well established that claims implicitly requiring particular characteristics in a biological material are allowable as long as the material is not already in the hands of the public — either by distribution, or by methods and materials for producing it. Knowledge of certain desirable features is not a bar to patentability if there is no way to make it using publicly available materials or information.

This is exemplified in *In re LeGrice* (133 USPQ 365 (CCPA 1962)). The question at issue was whether patents should be allowed claiming two particular strains of rose. One of the roses had been displayed in a plant catalog, along with a description of the genetic heritage and certain features. However, the roses had not been publicly sold. The court held that the roses were patentable, because the publications were incapable of placing the roses in the public domain by their descriptions when interpreted in the light of the knowledge possessed by plant breeders. Although plant breeders might produce roses using the same genetic parents, these

roses would not have the exact genetic makeup, and hence the exact phenotype, of the roses sought to be patented. The court considered and rejected the view that roses with similar but non-identical characteristics could be a bar to patentability. Granting of the patent placed the new roses in possession of the public, consistent with the public policy underlying patent law.

Applicants respectfully submit that Chatterjee et al. is a non-enabling reference, and as such, cannot be used as a prior art reference. As discussed in the interview, there are several reasons why the Chatterjee et al. reference is non-enabling:

(1) The very mechanism of antibody formation, coupled with an analysis of the nature of the amino acid sequence of the variable regions of 11D10[1] demonstrates that the probability of reproducing the amino acid sequence of 11D10 is vanishingly low.

(2) Sequence analysis of 11D10 demonstrates that it has the characteristics of a mature antibody that make it virtually impossible to reproduce without knowledge of its sequence.

(3) The sequence of 11D10 was not disclosed in any of the art of record in this application. Without knowledge of the sequence, prior publications are not enabling.

Mechanism of antibody formation. Antibodies are different from other mammalian proteins, in that affinity maturation results in a considerable reengineering of the heavy and light chain variable region amino acid sequences. This mechanism results in a wide diversity of amino acid sequences for antibodies with similar specificities. The possibility of two antibody-producing cell lines from different animals comprising exactly identical variable region genes *before mutation* is small; the possibility of two such cell lines comprising *identically mutated* variable region genes is so vanishingly small as to be essentially nil.

This section outlines what is known in the art about the generation of antibody diversity (for a classic review, see Tonegawa (1983) *Nature* 302:575.) Immunoglobulin heavy chain

[1] As discussed below, the sequence of 11D10 was not published anywhere prior to the filing date of provisional applications 60/031,306 (filed December 20, 1995) or 60/035,345 (filed January 26, 1996), to which the present application claims the benefit of priority.

genes arise in the B cell lineage from rearrangement of about 25-200 variable region genes, about 10 D regions, and about 5 J regions, with the order of 100 splice variants being possible for each V-D-J combination. Except for the lack of a D region, formation of a complete light chain gene is nearly as complex. Different heavy chains may associate with different light chains. The total number of combinatorial possibilities is therefore well over 10^8.

Only a proportion of these combinatorial possibilities yield viable antibody molecules with a particular specificity. However, another level of diversification is introduced following gene rearrangement. Antibody-producing B cells which are specific for an immunogen undergo *further* diversification by deliberate *somatic mutation* of the rearranged heavy and light chain variable region genes (reviewed, e.g., in Kochs et al. (1989), Ann. Rev. Immunol. 7:537; Berek et al. (1988), Immunol. Rev. 105:1). Mutation is believed to occur at a rate of about 10^{-5} per base pair per generation at the pre-B cell stage. It increases to nearly 10^{-3} per base pair per generation (almost one mutation per cell per generation) between 7 and 14 days following antigen exposure, when selected clones begin to enter the memory compartment. Some of the results of this mutation process are the emergence of clones with modified specificity, higher affinity, faster forward rate constants, and combinations thereof. However, mutations may occur anywhere within the entire length of the heavy and light chain variable region, including regions well outside the antigen-binding site. At the DNA level, even 5' and 3' untranslated regions of the DNA may be mutated.

In addition, it is clear that there are a vast number of possible antibody sequences that bind any particular antigen. When two different animals are immunized with the same antigen, they may each produce antibodies against it, but the antibody molecules obtained represent different solutions to the requirements of an antigen binding site. Accordingly, different antibody-producing cell lines cloned from a response against the same antigen will have substantially different variable region sequences.

This is illustrated in various experiments in which panels of monoclonal antibodies have been produced using haptens as the immunizing antigen. Haptens are small chemical groups, comprising only a single epitope. Yet, the antibodies raised against them have widely diverse amino acid sequences. Illustrative studies by Nahmias et al., Stenzel-Poore et al., Blier et al. and Leahy et al. are outlined in Appendix A to this paper, and exemplified in Figures 1 and 2 from Leahy et al.

These experiments illustrate that gene selection, gene splicing, and somatic mutation all contribute to widely diverse sequences that may be produced against any particular antigen. Somatic mutations accumulate as B cells pass through the memory compartment. Amino acid substitutions may occur at nearly any position in the light and heavy chain variable regions, as long as the replacement does not impair specificity. Antibody produced by a clone that has gone through the memory compartment comprises a number of such substitutions. The number of possible sequences for an antibody of any particular specificity is immense.

The steps of gene selection, gene splicing, and somatic mutation all occur independently of antigen. What creates the specificity of the antibody response is the preferential *selection* of clones of the desired specificity by antigen by interacting with antibody-producing cells *already expressing the assembled antibody at the cell surface.* Accordingly, the clones selected by antigen incorporate two types of changes: a) *those that enhance binding to antigen*; and b) *those that have a neutral effect on antigen binding.* This also indicates the importance of the selection process in determining the amino acid sequence of the antibody eventually chosen.

For changes that enhance binding to antigen, there are a large number of possible alternatives, both as to the amino acid used, and their location within the variable region. It is unusual that a particular amino acid mutation is absolutely required without the possibility of an alternative in order to bind directly to antigen. Mutations that enhance binding are generally found elsewhere within the variable region, and act to stabilize the preferred antigen-binding

pocket (reviewed recently in Patten et al.; see also Strong et al.). Presumably, all substitution or combination of substitutions that generate comparable degrees of stability to the antigen binding pocket will serve the purpose, and would therefore be equally selectable by antigen.

Changes that have a neutral effect on antigen binding will necessarily occur during the somatic mutation process, which is known to occur throughout the variable region gene (and into neighboring untranslated regions). Many of the mutations occurring outside the antigen-binding regions, or outside the complementarity-determining regions (CDRs), probably come within this category. Strong et al. found that only 6 of 16 somatic mutations in a monoclonal antibody contributed to the enhancement of affinity to the antigen, anti-*p*-azophenylarsonate. Blier et al. provide experimental evidence that changes non-deleterious to binding of the antigen NP accumulate during affinity maturation. They found that even after gene rearrangement and an initial round of somatic mutation, various sub-lines from a common ancestor appeared to accumulate additional mutations while retaining similar antigen-binding properties. Changes unrelated to antigen binding are especially hard to regenerate, since they are neither antigen-driven nor antigen-selected.

The hapten experiments outlined in Appendix A provide empirical demonstrations of how different antibodies with the same specificity from the same strain of mice may be. For example, the twelve anti-DNP spin label antibodies of Leahy et al. (Appendix A, Figures 1 & 2) showed an average variability of about 4 alternative amino acids at 83% of the positions in the amino acid sequence.

Uniqueness of the 11D10 sequence. An analysis of 11D10 demonstrates that it has the characteristics of a mature antibody that make it virtually impossible to reproduce without knowledge of its sequence.

The 11D10 monoclonal antibody was developed by immunizing and selecting with the murine monoclonal antibody MC-10, also denoted BrE1 (specification: page 99, lines 9-10).

Four immunizations were required to obtain a response (specification: page 99, lines 18-19), as opposed to the usual requirement of two immunizations for a mature anti-hapten response. Therefore, the clone emerging with anti-MC-10 specificity had been through the memory compartment and subjected to a period of somatic mutation at least once, and possibly three times. The number of possible sequences for an anti-idiotype antibody with specificity for MC-10 is expected *a priori* to be at least as large as described in the previous section for an anti-hapten antibody.

The extensive role of somatic mutation in generating the 11D10 sequence may be confirmed by comparing the amino acid sequence data (Figures 1 and 2 of the specification) with other known antibody sequences. Such a comparison is shown in the specification on page 117. The declaration of Dr. Sunil Chatterjee, an inventor on this application, accompanies this response. The declaration presents the results of the comparison using the BLAST algorithm to search GenBank.

As stated in Dr. Chatterjee's declaration, the comparison reveals the following:

- The 11D10 *light chain* variable region differs from the most closely matched previously known antibody sequences by at least 8 and more typically 10 or more substitution differences.
- No antibody was found using the same *heavy chain* V-D-J gene combination, indicating that the V-D-J splice employed in 11D10 is unusual.
- Antibodies apparently using the same *heavy chain* V gene element as 11D10 differed from 11D10 within this region by at least 13 substitutions and more typically by at least 16 substitution differences.

Figure 26(C) also provides a consensus analysis of the most closely matched sequences. The consensus sequence represents a prototype of the rearranged VJ light chain and VDJ heavy chain germ line sequences that were subsequently mutated to give the mature sequence found in

11D10. As stated in Dr. Chatterjee's declaration, the 11D10 amino acid sequences differ in 7 positions from the prototype light chain variable region, and at least 11 positions from the heavy chain variable region. Accordingly, it is likely that at least about 18 mutation events occurred in the generation of 11D10, of which 9 are outside the CDRs.

Appendix B attached to this amendment provides a calculation for the number of possible antibody molecules that are as extensively mutated as 11D10 and capable of binding the same antigen. *Even with conservative assumptions, the number of molecules is of the order of* $5.4 X 10^{35}$. Gene selection, splicing, and somatic mutation all contribute to this range. A major contributing factor is the wide variation in the number of locations that possible mutations (including those irrelevant to antigen binding) may occur within the sequence. Only about ten percent of the approximately 1300 primary fusion wells cultured from the immunized mice used by the inventors produced antibody that were positive in the first level of screening. Because so few specific cells can be identified, fused, and expanded from each immunized mouse, *it would take at least about* $5 X 10^{28}$ *mice to obtain a hybridoma line producing such a rare antibody molecule.*

The argument being put forward here does not depend on the exactness of the probability calculation. It is Applicants' position that any antibody that would require a very large number of mice to reproduce, combined with the extensive fusion, culturing, screening, cloning and competition-testing that is subsequently required, is beyond the scope of reproducibility in a second animal in any practical sense.

In summary, in view of the extensive mutation in 11D10, it is in all practical terms impossible for anyone to obtain 11D10 by immunizing a second animal, even using the exact protocol used to obtain 11D10.

The sequence of 11D10 was not disclosed. Neither the amino acid sequence for 11D10 nor the cDNA sequence encoding 11D10 was disclosed in the art of record. Because the

sequence of 11D10 is essentially not reproducible by immunizing naïve mice according to previously published methods, one skilled in the art would have to have the amino acid or DNA sequence information for the variable region of 11D10 in order to practice the invention, or else have access to the 11D10 antibody producing cell line.

(b) No disclosure of or public access to 11D10 or the hybridoma producing 11D10

As discussed above, neither the amino acid sequence nor any polynucleotide sequence encoding 11D10 (variable regions) was disclosed the prior art of record. In addition, neither 11D10 nor the hybridoma producing 11D10 have been made accessible to the public before filing the priority applications (or the present application).

Attached to this response is a declaration by Dr. Malaya Chatterjee, one of the inventors, who states that the 11D10 producing cells, the re-cloned 11D10 cell lines, the predecessors and progeny of these cells and cell line, and the antibody produced by these cells and cell line has remained under the strict and exclusive control of herself and other inventors. Dr. Chatterjee also states that there has been no free exchange of 11D10 or the cell line producing 11D10. Neither 11D10 nor the cell line producing 11D10 were made available to the public prior to the filing of this application (as well as previously filed provisional application 60/031,306, filed December 20, 1995), and both remain under her strict supervision and control. Further, as requested by the Examiner, Dr. Chatterjee provides information regarding co-authors of 11D10 publications (papers (a) through (f), as enumerated above). She states that, to the best of her knowledge and belief, none of the co-authors who worked in her laboratory had possession of 11D10 or the 11D10 producing cell line after leaving her laboratory, nor did they distribute 11D10 or the 11D10 producing cell line outside the laboratory while they were affiliated with the laboratory. With respect to co-authors Drs. Ceriani and Kohler, who were not affiliated with Dr. Chatterjee's laboratory, Dr. Chatterjee states that, to the best of her knowledge and belief, neither had ever had possession of 11D10 or the 11D10 producing cell line.

Also attached is a declaration of co-inventor Dr. Ken Foon who states that the clinical trials involving 11D10 were conducted under his exclusive direction and strict supervision, with medical staff permitted access to 11D10 only under his strict and exclusive control, and solely for purposes of administration to participants of the clinical trial. Dr. Foon also states that the participants were only allowed access to 11D10 by injection and were not able to distribute 11D10. In terms of what participants knew about 11D10, they were only told that 11D10 was an anti-idiotype antibody, with no information provided about the amino acid sequence or DNA coding sequences for 11D10.

Dr. Sunil Chatterjee (a co-inventor) also states in his declaration that the 11D10 producing cell line was maintained in his laboratory under his strict and exclusive control. Dr. Chatterjee additionally states that, to the best of his knowledge and belief, no one in his laboratory took 11D10 or the 11D10 producing cell line from his laboratory, and nor did anyone have permission to do so.

Summary

Because of the uniqueness of the 11D10 antibody and the virtual impossibility of regenerating it in a second animal, the invention can only be practiced by either: (a) obtaining the antibody (or the antibody encoding gene) from the 11D10 producing hybridoma cell line; or (b) synthetically producing an antibody with identical amino acid sequences, based on the 11D10 sequence data. The invention cannot be said to be in possession of the public at the time of filing of the priority application(s). The references of record do not provide sufficient disclosure for one skilled in the art to reproduce 11D10.

Applicants respectfully request that the Office acknowledge on the record that there are no rejections under § 102 (a) or (b).

Inventive entity

During telephone interviews on July 14 and September 25, 1998, and the Examiner raised a question of inventive entity with respect to co-authors of the references (a) through (f) enumerated above.

Accompanying this amendment is a declaration by Dr. Malaya Bhattacharya-Chatterjee, one of the inventors of this application. Dr. Chatterjee explains the roles of the co-authors with respect to generation or selection of the 11D10 antibody or the cell line producing 11D10. She states that the co-authors who contributed in any way to the generation of 11D10 antibody or the 11D10 producing cell line (Drs. Mrozek, Mukerjee, and Chakraborty) were all working under her direct supervision, and did not make any independent contributions with respect to generation of 11D10 antibody or the 11D10 producing cell line. With respect to the remaining co-authors (Drs. Ceriani, Kohler, and Sherrat), Dr. Chatterjee states that they did not make any contributions to generating 11D10 antibody or the 11D10 producing cell line.

Accordingly, Applicants believe that none of the co-authors made an inventive contribution toward the invention claimed in this patent application, and should not be named as co-inventors. Applicants respectfully request that the Office acknowledge on the record the lack of any rejections under § 102(f).

Non-access to 11D10 or the hybridoma producing 11D10

The lack of availability of the 11D10 antibody and the hybridoma producing 11D10 before the priority date(s) of this application was discussed above, but for convenience will be summarized here.

Inventors

The declarations of the inventors Malaya Chatterjee, Kenneth Foon, and Sunil Chatterjee all state that that both the 11D10 antibody and the hybridoma producing the antibody were kept under their strict and exclusive control.

Co-author non-inventors

The Examiner has raised a question with respect to the co-authors of the papers cited above regarding distribution of the 11D10 antibody or the hybridoma producing 11D10. Dr. Malaya Chatterjee's declaration addresses this issue.

With respect to co-authors Drs. Mrozek, Mukerjee, Chakraborty, and Sherratt, Dr. Chatterjee states that, to the best of her knowledge and belief, they did not have possession of the antibody or hybridoma after leaving her laboratory, and nor did they distribute 11D10 or 11D10 producing cell line while they were in her laboratory. With respect to co-authors Drs. Ceriani and Kohler, Dr. Chatterjee states that, to the best of her knowledge and belief, neither had ever had possession of 11D10 antibody or the 11D10 producing cell line.

Applicants respectfully request that the lack of public availability of 11D10 and the 11D10 producing cell line be acknowledged by the Office on the record.

35 U.S.C. § 103

The Examiner has not raised any issues under 35 U.S.C. § 103. There should be no such issues, given that there is art under § 102 (see discussion above), and the only other remaining references (which would be secondary) are generalized papers which describe, for example, how to generate an antibody.

Applicants respectfully request that the Office acknowledge on the record that there are no § 103 rejections.

Applicants believe that all issues raised by the Examiner have been addressed and resolved in accordance with the Examiner interviews and respectfully request allowance of the pending claims. Applicants' representative also invites the Examiner to call at the number below to discuss any questions or issues pertaining to this response.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952**. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Dated: October 8, 1998

Respectfully submitted,

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